IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: Philippe ROUANET *et al.*

Title: **PREVENTION AND TREATMENT OF BREAST CANCER WITH 4-HYDROXY TAMOXIFEN**

Appl. No.: 10/734,638

Filing Date: 12/15/2003

Examiner: Abigail Manda Cotton

Art Unit: 1617

Confirmation Number: 9056

DECLARATION OF VALERIE MASINI-ETEVE, PH.D. UNDER 37 C.F.R. § 1.132

Mail Stop Amendment
Commissioner for Patents
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Sir:

I, Valérie Masini-Etévé, do hereby declare and state as follows:

1. I am employed by Laboratoires Besins-International, the assignee of the captioned U.S. patent application, and understand that this declaration may be used to support the application.

2. My position at Laboratoires Besins-International is Head of Non Clinical R&D. I joined Laboratoires Besins-International in 1993 as a researcher working on formulations and in vitro percutaneous absorption. I received a Ph.D. in Cutaneous Biology and Pharmacology from the University of Paris XI in 1995. My responsibilities at Laboratoires Besins-International expanded in 2003 to include non-clinical, in vivo, research and development.

1. **Penetration Enhancement of 4-Hydroxy Tamoxifen with IPM**

3. I submitted a previous declaration (executed September 10, 2007) in the application which presented data related to the penetration enhancement of 4-hydroxy tamoxifen (4-OHT) with isopropyl myristate (IPM). I attended a Patent Office Interview on November 14, 2007, where the Examiners questioned the ability of IPM to enhance penetration at concentrations greater than 1.0%.

4. I conducted or oversaw the following in vitro permeation experiments to assess the affect of IPM on 4-OHT penetration.

5. Hydroalcoholic gel preparations comprising 0.228% (w/w) radiolabeled (3H) 4-OHT were prepared in accordance with Example 1 of WO 06/040196, with the alcohol content slightly increased (73.9% versus 70.9%) to solubilize the higher IPM concentrations. The preparations comprised 0%, 1.5%, or 2.0% (all w/w) IPM. One black and one mixed race human abdominal skin samples (dermatomed to ±350μM) were used in the experiments. The experiments were carried out in accordance with standard procedures, outlined below.

6. In vitro transdermal absorption is quantitatively studied on human ventral dermatomed biopsies placed in a static diffusion cell (Franz cell). A dermal biopsy is maintained horizontally between two parts of the cell, thus delimiting two compartments:

   The upper compartment (epidermal) is made of a glass cylinder, typically having a precisely defined area of 1.77 cm2, and is placed on the upper side of the skin

   The lower compartment (dermal) is applied to the lower face of the tegument, and comprises a reservoir of fixed volume and a lateral collection port.

   The two compartments are assembled via a clamp.

   The lower compartment (dermal) is filled with a receptor liquid (typically sodium chloride supplemented with bovine serum). At each time point tested, the receptor liquid is entirely sampled out by the lateral collection port and replaced by fresh

liquid. The lower compartment is thermostated to 37°C. Homogeneity of the temperature and the content in the receptor fluid is maintained by stirring (magnetic stirrer).

The upper compartment (epidermal) is open, exposing the epidermal surface to laboratory air.

7. For each formulation tested, 10 Franz cells were set up, with the 2 different donor skin samples distributed between the 10 cells. Ten µl of gel were applied with a micropipette over the entire surface of the epidermis delimited by the glass cylinder of the Franz cell. Sampling from the liquid contained in the dermal compartment was carried out via the lateral collection port up to 24 hours post-application. At the end of the experiment, residual drug remaining at the surface of the skin was removed by washing the skin surface with soapy water and then rinsing. The application area was then wiped with a cotton swab. All washing media, the cotton swab and the upper part of the Franz cell were introduced into a flask with about 45 ml of ethanol, precisely weighed, and incubated overnight at room temperature in order to extract residual radioactivity. The epidermis was separated from the dermis by gentle scraping with a scalpel, and the dermis was separated from the lower part of the Franz cell. The epidermis and dermis were digested for a few hours at 60°C with 1 ml and 3 ml of Soluene 350™ (Packard), respectively, to extract radioactivity.

8. Radioactive samples were prepared for analysis as follows:

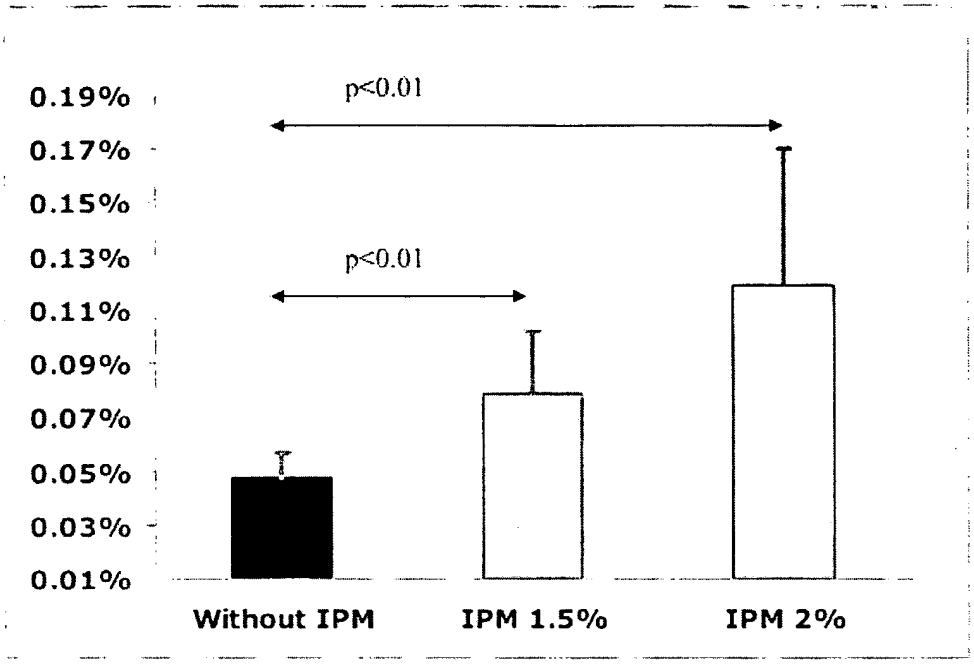
(1) The receptor liquid sampled from the lower compartment of each Franz cell was incorporated into 15 mL of liquid scintillation cocktail (Picofluor 40R, Packard).

(2) A precisely weighed aliquot of the solution containing the washing solvents was mixed with liquid scintillation cocktail Picofluor 40R.

(3) 15 ml of the liquid scintillation cocktail Hionic FLuorR were added to each sample of digested epidermis and dermis.

9. Radioactivity was measured by liquid scintillation using a Packard-tricarb 2900 TR particle counter. Results are expressed in weight or percentage of substance found in the samples with respect to the administered amount.

10. The percentage of radioactive 4-OHT recovered in the receptor liquid after 24 hours is shown below for each formulation. The average from 10 individual samples of each formulation is reported with the corresponding standard deviation shown by the bars. The p-value for improved enhancement versus the "Without IPM" results is < 0.01 for each concentration tested.



11. The results show that the concentrations of IPM tested have a significant impact on penetration. Thus, IPM is an effective penetration enhancer for 4-OHT when used at concentrations above 1.0%, including at 1.5% and 2.0%.

12. I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful, false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that willful, false statements may jeopardize the validity of the application or any patent issued thereon.

21 February 2008
DATE

Masini-Etévé
Valérie Masini-Etévé, Ph.D.